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BREAKTHROUGHS AND VIEWS

β-1,4-Galactosyltransferase and Lactose Synthase: Molecular Mechanical Devices

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Recent structural investigations on the β-1,4-galactosyltransferase-1 (Gal-T1) and lactose synthase (LS) have revealed that they are akin to an exquisite mechanical device with two well-coordinated flexible loops that are contained within the Gal-T1 catalytic domain. The smaller one has a Trp residue (Trp314) flanked by glycine residues. The larger one comprises amino acid residues 345 to 365. Upon substrate binding, the Trp314 side chain moves to lock the sugar nucleotide in the binding site, while the large loop undergoes a conformational change, masking the sugar nucleotide binding site, and creates (i) the oligosaccharide binding cavity; (ii) a protein–protein interacting site for the enzyme's partner, α-lactalbumin (LA); and (iii) a metal ion binding site. Only in conformation II do Gal-T1 and LA form the LS complex, enabling Gal-T1 to choose the new substrate glucose. LA holds and puts Glc right in the acceptor binding site of Gal-T1, which then maximizes the interactions with Glc, thereby making it a preferred acceptor for the LS reaction. The interaction of LA with Gal-T1 in conformation II also stabilizes the sugar–nucleotide–enzyme complex, kinetically enhancing the sugar transfer, even from the less preferred sugar nucleotides. The conformational change that masks the sugar nucleotide binding site can also be induced by the acceptor alone, thus making it possible for the protein to act as a specific lectin.

Key Words: **mechanical device; glycosyltransferases; β-1,4-galactosyltransferase; α-lactalbumin; lactose synthase; conformational change; lectin.**

GALACTOSYLTRANSFERASES

A superfamily of enzymes called glycosyltransferases synthesizes complex carbohydrates of glycoconjugates of cells by transferring a sugar moiety of a sugar nucleotide to an acceptor sugar (1, 2). The galactosyltransferase family, in the presence of metal ion, transfers galactose from uridine-diphosphate-D-galactose (UDP-Gal) to an acceptor sugar molecule. To date, three subfamilies, β1-4-, β1-3-, and α1-3-, have been well characterized (3) and they generate β1-4-, β1-3-, and α1-3- linkages between galactose and the acceptor sugar, respectively. Cloning has identified the presence in each family of several members that have sequence homology within the family members (3). The β1-4-galactosyltransferase (Gal-T) family, which was the first one to be cloned (4–6), consists of at least seven members, Gal-T1 to Gal-T7 (3), with a 25 to 55% sequence homology. These enzymes are expressed in different tissues and show differences in the oligosaccharide acceptor specificity (7, 8).

INTERACTIONS WITH α-LACTALBUMIN (LA) MODULATE THE ACCEPTOR AND DONOR SPECIFICITY OF β-1, 4-GALACTOSYLTRANSFERASE (Gal-T1)

LA Modifies the Acceptor Specificity of Gal-T1

In the mammary gland, only Gal-T1 is expressed (9) and it interacts with the calcium binding protein, α-lactalbumin (LA), that is expressed in the mammary gland during lactation, to form the lactose synthase complex (LS). This complex transfers galactose moiety to free glucose to produce lactose (10) (Fig. 1A), the major carbohydrate component of milk. LA interacts with Gal-T1 and lowers the K_m for glucose 1000-fold (11) by promoting the binding of Glc to the enzyme and thereby altering the sugar acceptor specificity. In the

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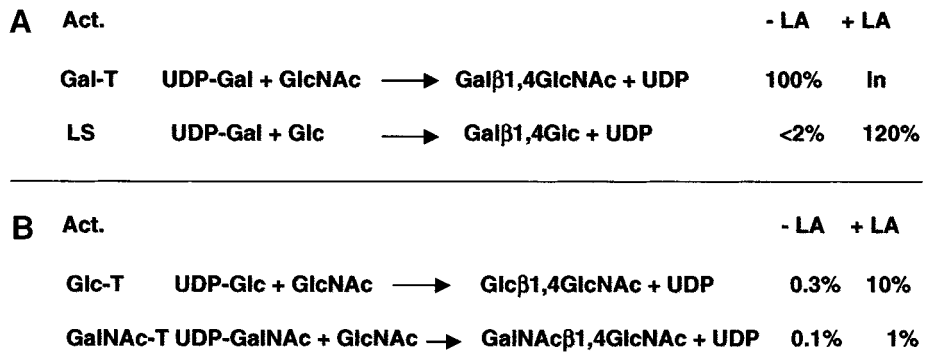


FIG. 1. (A) Modulation of the sugar acceptor specificity by LA. (B) Modulation of the sugar donor specificity by LA. Galactosyltransferase activity (Gal-T); lactose synthase activity (LS); glucosyltransferase activity (Glc-T); N-acetylgalactosaminyltransferase (GalNAc-T).

absence of LA, glucose (Glc) is a poor substrate for β4Gal-T1 (K_m for glucose ~2 M). Cross-linking experiments have shown that the two proteins, LA and Gal-T1, interact only in the presence of the Gal-T1 substrate. These interactions are specific (12, 13). Lysozyme, with which LA shows extensive homology and which has a similar 3-D structure, cannot replace LA. Besides Gal-T1, some other family members of Gal-T also interact with LA, resulting in different effects (14). LA also interacts with the N-acetylgalactosaminyltransferase from *Lymnaea stagnalis* (snail) and from bovine milk, and modulates their activity to accept Glc as an acceptor (15). On the other hand, N-acetylglucosaminyltransferase of snail, which has a high sequence similarity to the mammalian β1-4-galactosyltransferases (16), does not interact with LA (17). These observations suggest that the LA interacting site of Gal-T1 is present in other related glycosyltransferases, and that even though LA is not expressed in snail, the interacting site has been preserved during evolution.

LA Influences the Sugar Donor Specificity of Gal-T1

Although the predominant reaction of Gal-T1 is that it transfers Gal from UDP-Gal to GlcNAc, it also transfers, albeit at a much lower efficiency, Glc, 2-deoxy-Glc, arabinose, and GalNAc from their respective UDP derivatives to GlcNAc (18) (Fig. 1B). LA enhances these transfer reactions between 10- and 30-fold, depending upon the sugar nucleotide used (19, 20). The transfer of Glc from UPD-Glc is enhanced about 30-fold, corresponding to about 10% of Gal-T activity (19). On the other hand, GalNAc transfer from UDP-GalNAc is enhanced only 10-fold, corresponding to 1% of Gal-T activity (20).

STRUCTURE-BASED EXPLANATION OF Gal-T1 PROPERTIES

Comparison of the crystal structures of Gal-T1, with (13) and without substrates (21) and in the LS complex (13), shows that Gal-T1 upon ligand binding, undergoes a large conformational change from I to II, creating the acceptor binding pocket, and thereby converting the enzyme from a native inactive state to an active state. The structures reveal that Gal-T1 has two flexible loops which play crucial roles in the functioning of the enzyme. First, it has a short flexible loop sequence with a Trp residue at position 314 that is flanked by Gly residues [GWGG (313–316)], allowing a rotary motion for the movement of the Trp side chain. A second larger flexible loop region, comprising residues 345 to 365 (Fig. 2A, conformation I), contains residues essential for interacting with the substrates. UDP-Gal with Mn^{2+} binds to Gal-T1 only in conformation I, and upon its binding the Trp314 side chain moves from facing outward to facing into the catalytic pocket, forming a hydrogen bond with the nucleotide sugar (13). The second larger flexible region, residues 345 to 365, changes its conformation (Figs. 2B and 3), repositioning residue His347 in such a way that it can participate, along with the Asp 254, Met344, and the phosphates of UDP-Gal, in the coordination of a metal ion (Fig. 2B). Residues 352 to 355 of this loop form a turn, while residues 359 to 365 form an α-helical secondary structural element that is important for the binding of the N-acetyl group of GlcNAc. Residues Ile345 and His365, flanking the large flexible region, appear to act as hinges that enable the region to undergo the conformational change from I to II. The conformational change locks the UDP-Gal in the binding pocket and its dissociation is hindered. In the double substrate kinetic analysis, the parameter K_{ia} reflects the dissociation of UDP-Gal (Fig. 3). In the galactosyltransferase and lactose synthase reactions, the value for K_{ia} is zero (22), which is consistent with the trapping of UDP-Gal substrate by the conformational change. Since the sugar binding site is created only after the conformational change has occurred, the sugar acceptor can next bind to the site. Thus, the enzyme kinetics have to follow a sequential ordered mechanism (22). If the ac-

ceptor induces the conformational change to II, prior to the binding of UDP-Gal, then the sugar nucleotide binding site is closed and the molecule will not function as an enzyme but as a lectin. Thus, the enzyme catalysis has to follow a sequential ordered rather than a sequential random mechanism (22, 23).

CONFORMATIONAL CHANGE REDEFINES THE CATALYTIC POCKET OF Gal-T1

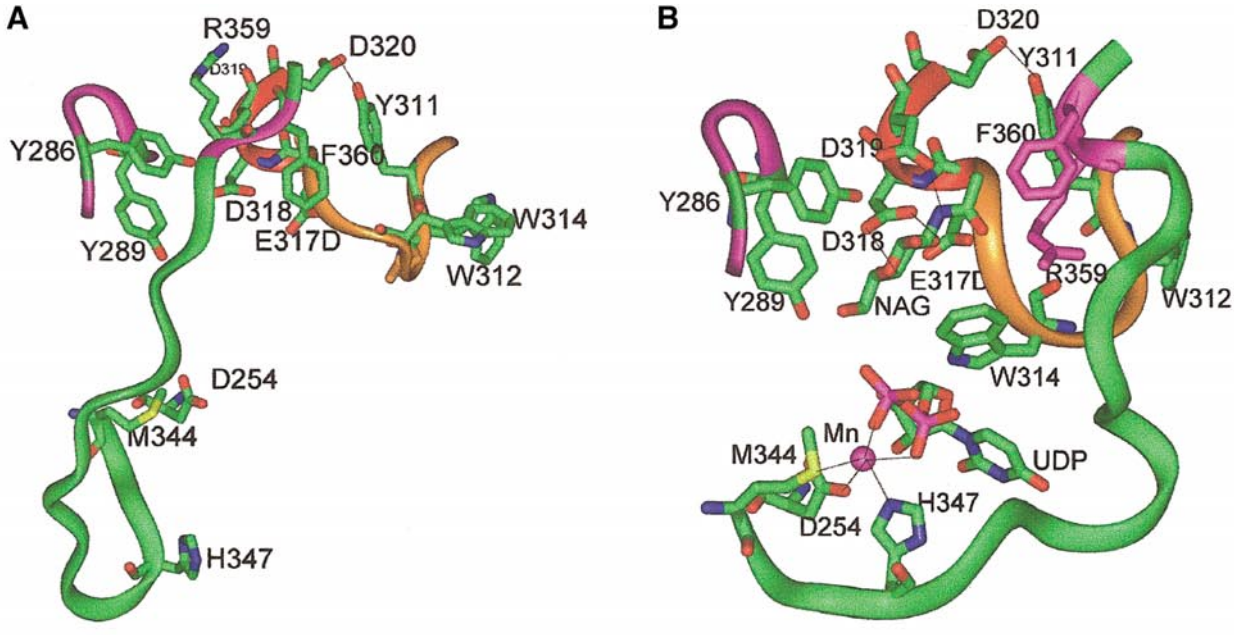
The conformational change, from the native state (apoenzyme, conformation I) to the substrate bound state, conformation II, simultaneously creates the sugar and LA binding sites on Gal-T1, and covers a part of the UDP-Gal binding site that results in the formation of a full catalytic pocket in Gal-T1, converting the enzyme from an inactive to an active state. Several protein regions lying buried in conformation I (21), are exposed in conformation II (13). The native Gal-T1 structure is a cone-shaped single domain protein containing a 13-Å-wide pocket. In conformation II it is seen to be a two-domain protein, α- and β-domains, with the substrate binding site located between these two domains, which extends from one end to the other end of the molecule. With the redefinition of the catalytic pocket, the mutational data that could not be explained on the basis of conformation I can now be successfully explained. For example, the mutation of Trp314 was found to abolish the catalytic activity of Gal-T1 (24). In conformation I, Trp314 was found outside the catalytic pocket, and did not display any direct interaction with the donor or acceptor molecules (21). In the active conformation II, Trp314 was found to reside inside the catalytic pocket (Figs. 2B and 3) and was involved in interactions with both the UDP-Gal and the acceptor molecule. Trp314 in this orientation is the most likely Trp residue that was observed previously to be protected from destruction by UV irradiation when UDP-Gal and Mn^{2+} were present (25). Similarly, Phe280 and Phe360 residues seem to affect the binding of GlcNAc to Gal-T1. These two residues interact directly with the GlcNAc molecule in conformation II; hence, mutation of these residues is expected to affect the binding of GlcNAc (26). In conformation I these residues are 14 Å apart and the acceptor binding site is only partially present (Fig. 2A). Similarly, Asp318 and Asp319 form hydrogen bonds with GlcNAc, and mutating either of these residues abrogates the enzymatic activity (27, 28). Asp320 is hydrogen-bonded to Tyr311, which potentially stabilizes the loop structure. Mutation of Asp320 inhibits the binding of GlcNAc (27). Thus, the redefinition of the catalytic pocket seems to fit all the mutational data on Gal-T1.

Gal-T FAMILY MEMBERS CAN BEHAVE AS SPECIFIC LECTINS

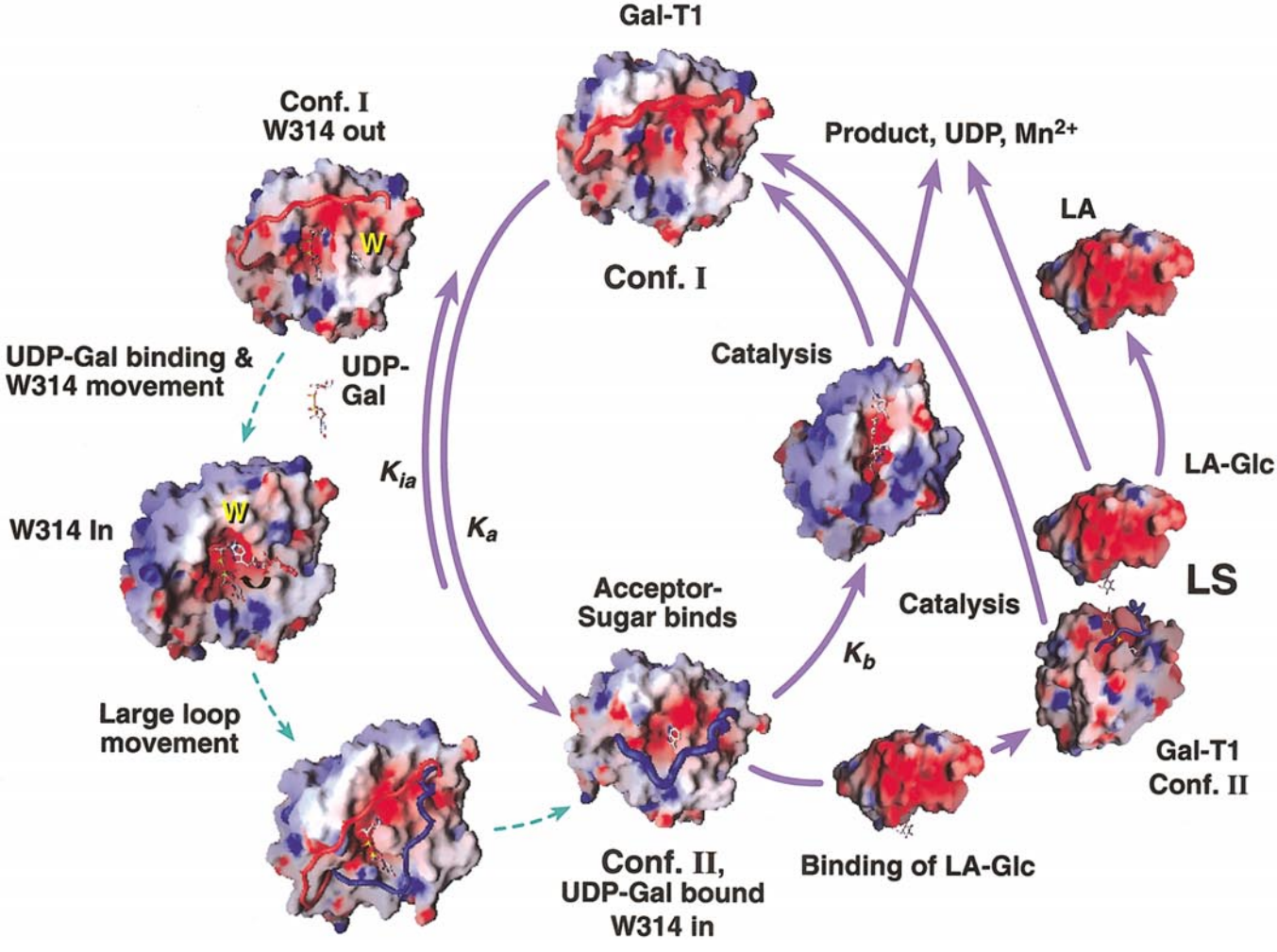
The sugar acceptor alone can also induce the conformational change in Gal-T1, as evidenced by the crystal structure of Gal-T1 · LA · GlcNAc (13). It requires, however, millimolar concentrations of the sugar, whereas UDP-Gal induces the conformational change just in micromolar concentrations. In the presence of GlcNAc alone, the orientation of the side chain of Trp314 changes to make hydrophobic interactions with the GlcNAc molecule, while the large loop changes its conformation, creating not only the GlcNAc binding pocket but also a site that can bind the extended sugar acceptor, its natural substrate. The sugar binding site can accommodate an N-glycan, pentasaccharide in length. The 1-3 and 1-6 arm of a biantennary oligosaccharide with GlcNAc as terminal residues, can be docked in the extended sugar binding site without any steric hindrance. Sequence comparison of the human Gal-T family members shows sequence variations in their oligosaccharide binding site that would account for their respective preferences for different oligosaccharides (14, 29). Thus, in the absence of a sugar nucleotide, the conformational change in the large loop puts a lid—a cover—on the sugar nucleotide binding site, while simultaneously creating the sugar binding site that enables the protein to act as a specific lectin.

CONFORMATIONAL CHANGE IN Gal-T1 CREATES A BINDING SITE FOR LA

Most of the residues of Gal-T1, involved in the interaction with LA, are buried under the large flexible loop in conformation I and thus prevent its interaction with LA. The two molecules cannot be cross-linked in the absence of substrates (12, 13). Substrate-induced conformational change on Gal-T1 exposes these residues and repositions residues Arg359 to Ile363 in the α6 helix of Gal-T1. This helix interacts both with LA and GlcNAc (13), which explains why LA and Gal-T1 can be cross-linked only in the presence of the substrate (12, 13). Interactions between LA and Gal-T1 in conformation II mostly involve hydrophobic patches present in both molecules. The LA residues Phe31, His32, and some of the residues from the carboxyl terminal flexible region form the hydrophobic patch on LA and have previously been identified by mutational analysis (30). In the Gal-T1 residues, Phe280, Tyr286, Gln288, Tyr289, Phe360, and Ile363 form the hydrophobic patch (13), as well as the extended region of the monosaccharide binding site. LA and the oligosaccharides would be expected to compete for the same extended-sugar binding site on Gal-T1. No interaction between LA and the nucleotide sugar donor substrate molecule has been discerned.



2 CONFORMATION I CONFORMATION II



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MODULATION OF THE ACCEPTOR SPECIFICITY OF Gal-T1 BY LA

The crystal structure of Gal-T1 in its complex with LA and Glc provided the structure-based information on the modulation of the acceptor specificity of Gal-T1 by LA (13). By making a hydrogen bond between the O1 hydroxyl group of Glc molecule and the N^{-1} nitrogen atom of His32, LA holds the Glc molecule in the monosaccharide binding pocket of Gal-T1 (Fig. 3). For the binding of GlcNAc in the monosaccharide binding site, there is a hydrophobic N-acetyl group-binding pocket, formed by residues Arg359, Phe360, and Ile363. The Glc molecule, which lacks the N-acetyl group, will bind only very weakly in this binding pocket (13). While interacting and associating with Gal-T1, LA holds the Glc molecule in the acceptor binding site that modulates itself by adjusting the orientation of the side chain of Arg359, thus closing the hydrophobic N-acetyl group-binding pocket and maximizing its interactions with Glc. This way Gal-T1 modulates the GlcNAc binding site itself and broadens the acceptor binding specificity.

LA PLAYS A KINETIC ROLE IN THE MODULATION OF THE SUGAR DONOR SPECIFICITY OF Gal-T1: KINETIC ROLE OF LA IN GLUCOSYLTRANSFERASE ACTIVITY OF Gal-T1

Since LA does not make any contact with the nucleotide donor in the Gal-T1-sugar nucleotide complex, its role in stimulating the transfer of Glc from UDP-Glc to GlcNAc by Gal-T1 is at the kinetic level and not the result of interactions between UDP-Glc and Gal-T1 (19). Although the structure of Gal-T1 bound with UDP-Glc is quite similar to that bound with UDP-Gal, there are a few differences. The O4 hydroxyl group is equatorial in Glc, whereas it is axial in Gal. As a result, the O4 hydroxyl oxygen of the Gal in the Gal-T1 · UDP-Gal · Mn^{2+} complex points toward the acceptor binding site (Fig. 2B) and forms a weak hydrogen bond with the Asp318 side chain carboxylate oxygen atom. In the Gal-T1 · LA · UDP-Glc · Mn^{2+} complex, although the O4 hydroxyl group of Glc is hydrogen-bonded to Glu317, it points away from the acceptor-binding site and Asp318 (distance 3.8 Å) (19). This makes UDP-Glc a weak donor, and because of weak interactions it does not induce the conformational change as efficiently as does UDP-Gal. The double substrate kinetic analysis of the glucosyltransferase (Glc-T) reaction in the absence of LA shows that UDP-Glc dissociates from the enzyme · UDP-Glc complex (K_{ia} = 20 μM), whereas in the Gal-T reaction, UDP-Gal does not dissociate from the enzyme · UDP-Gal complex (K_{ia} = 0) (19, 22). In the presence of LA, the data of double reciprocal plots showed essentially parallel lines, as in the Gal-T reaction. K_{ia}, the dissociation constant for UDP-Glc, is zero and the k_{cat} increases 30-fold. The presence of LA seems to stabilize the enzyme · UDP-Glc complex in conformation II, thereby enhancing the catalytic reaction.

WHAT ARE THE POSSIBLE BIOLOGICAL IMPLICATIONS OF THE CONFORMATIONAL CHANGE IN Gal-T1?

Lectin-Related Property

Gal-T1 can act as a lectin if the conformational change to II is induced by the binding of the sugar acceptor to Gal-T1, as has been observed in the crystal structure of Gal-T1 · LA · GlcNAc (13). Each member of the Gal-T family, whose expression has been shown to be tissue-specific (7) and which have preferences for specific oligosaccharides (8), can act as tissue-specific lectins. Gal-T1, has been shown to be expressed at the cell surface, and has been suggested to play a role in the cell-cell interactions (31). Since for it to function as an enzyme, the presence of sugar nucleotide at the cell surface has been questioned (32), we suggest that for Gal-T1 to act as a lectin, the presence of sugar-nucleotide is not required. The activity of Gal-T1 has been reported to be regulated by phosphorylation (33). Such modifications could induce conformational change in Gal-T1, leaving it in conformation II as enzymatically a dead molecule but with the potential to bind an oligosaccharide and act as a lectin. However, it remains to be determined if such a modification indeed plays any role in the conformational change of Gal-T1.

LA Binding Site-Related Property

In addition to Gal-T1, the LA binding site is also present on some other glycosyltransferases and has

FIG. 2. In Gal-T1 the small loop, comprised of residues $GW^{314}GG$, and the large loop, composed of residues 345 to 365, undergo a conformational change from I (A) to II (B). For comparison, Tyr286 and Tyr289 are shown as reference residues.

FIG. 3. Molecular dance of Gal-T1. During catalytic cycle, Gal-T1 in conformation I binds UDP-Gal and Trp314 moves from outside to the inside (W314 In) of the cavity (green arrow), making a hydrogen bond with the oxygen of phosphate that locks the sugar nucleotide. Movement of the large loop (red) changes the conformation (green arrow pointing to blue loop), which covers the bound sugar nucleotide (conformation II) and creates the sugar acceptor and LA binding sites. Either sugar can bind in the cavity generating Gal-T activity, or LA with Glc can bind to this site generating lactose synthase activity. After the products are released, the large loop reverts to conformation I, while Trp314 moves out of the catalytic pocket, releasing UDP and Mn^{2+}. Gal-T1 can go through another cycle, starting with conformation I. K_{ia} is the dissociation constant of the sugar nucleotide, and K_a and K_b represent the true K_m of sugar nucleotide and acceptor, respectively.

been conserved during evolution. For example, β-1,4-N-acetylgalactosaminyltransferases from *Lymnaea stagnalis* (snail) and bovine milk, which transfer GalNAc to GlcNAc, also interact with LA, which modulates their activity to accept Glc as an acceptor (15, 34). Also, other Gal-T family members in the mouse and in human species are expressed in various tissues where LA, the mammary gland-specific protein, is not expressed. This suggests that molecules like LA may interact with the LA binding site, which is also the oligosaccharide binding site, and may act as specific lectin inhibitors by competing for the binding of the oligosaccharide.

Gal-T6, a Lactosyl-Ceramide Synthase, May Require an LA-like Activator

It has been suggested that Gal-T6 is a lactosylceramide synthase that transfers Gal to the Glc moiety of glucosylceramide (35). A protein sequence comparison of Gal-T1 and Gal-T6 indicates that the GlcNAc binding residues that are present in Gal-T1 are also present in Gal-T6, and later also transfer Gal to GlcNAc (8). Since Glc cannot bind in the GlcNAc binding site, for Gal-T6 to transfer Gal to the Glc moiety of glucosylceramide, a modulation mechanism similar to the LS system has to exist. The modulation of the acceptor site in Gal-T6 may either be by the ceramide moiety alone or by a protein molecule that may function just as LA does in the lactose synthase reaction. A homology modeling study on Gal-T6 based on the Gal-T1 crystal structure indicates that the oligosaccharide binding site on Gal-T6 is quite similar to Gal-T1 and that this site does not have any hydrophobic pocket that would bind to ceramide. Therefore, we propose that glucosylceramide may be delivered to Gal-T6 by an activator protein, like the GM2-activator protein (GM2-AP) (35), which may utilize the LA binding site on Gal-T6 by holding the ceramide moiety of glucosylceramide while delivering the head group Glc in the monosaccharide binding-site of Gal-T6. Then the N-acetyl binding pocket of Gal-T6 may adjust to make maximum interactions with the Glc moiety, in a mechanism similar to the lactose synthase system.

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